THE RETICULO-ENDOTHELIAL APPARATUS OF ELEDONE CIRROSA

Angus E Stuart

THE RETICULO-ENDOTHELIAL APPARATUS OF *ELEDONE CIRROSA*

Angus E. STUART

Department of Pathology, University of Edinburgh

ABSTRACT

The reticulo-endothelial apparatus of *Eledone cirrosa* has been investigated by the intraveneous injection of colloidal carbon. Phagocytic cells were found in several glands. A primitive recognition system may exist in the gland of Hensen.

The purpose of this paper is to describe the distribution of phagocytic cells in the different organs of one species of octopus, *Eledone cirrosa*, and to assess whether this group of cells can properly be called a reticulo-endothelial system. The carbon used in these experiments has been specially prepared (BENACERRAF, BIOZZI, HALPERN and STIFFEL, 1957). When used in mammals this material is stable in serum, does not normally cross the capillary barrier and is non toxic for the reticulo-endothelial cells and the organism. The particles are homogenous in size and measure about 250 Å in diameter.

It is generally recognised that although the invertebrates have phagocytic or amoeboid wandering cells, they are probably immunologically inert and certainly do not respond to antigens in the same way as fish or mammals. The octopus is an extremely complex

Acknowledgements. — I am indebted to the Ciba Foundation and to Professor DRACH for their encouragement and help. I am especially grateful to Michel GALANGAU for valuable technical aid.

and highly developed invertebrate (YOUNG, 1964) and possesses a leucoformative centre variously called the gland of Hensen or Faussek, the white body or the " corps blanc ". Particular attention has been given in this paper to the phagocytic cells of this organ with the hypotheses in view that the function of this organ is immunological.

MATERIAL AND METHODS

ANIMALS

11 females of *Eledone cirrosa* weighing approximately 250 - 350 grams were used. Of these 9 were injected intravenously in one of the lateral arm veins with carbon. The animals were anaesthetised with a solution of urethane in sea water before injection and were killed with urethane at intervals of 3, 4 and 14 days after injection. The 2 uninjected animals served as normal controls.

CARBON

This was injected intravenously with a No. 19 hypodermic needle in small, intermediate and high doses as follows, a single injection of 1 ml., 2 consecutive daily injections of 1 ml., and 2 daily consecutive injections of 2 ml. The concentration of carbon was 16 mgs/100 ml.

HISTOLOGY

Small pieces of tissue were placed in Halmi's fixative and paraffin sections stained with haematoxylin and eosin. The following organs were examined. Liver, muscle, posterior salivary gland, anterior salivary gland, eye and gland of Hensen, gills, branchial heart, gill spleen, skin. Blood smears were air dried and stained by Leishman's method. The nomenclature of ISGROVE (1903) has been used.

RESULTS

The carbon was well tolerated in all doses. During the intravenous injection the carbon spread backwards into the small lateral radicles and simultaneously the veins of the arms became very black quite suddenly. When the animal had recovered from the anaesthetic and the arms were firmly adherent to the plastic aquarium one could clearly observe the superficial carbon filled veins. These vessels pulsed rhythmically, segments alternately filling with carbon and then emptying, giving the impression of black streaks followed by clear. At 7 hr. the concentration of circulating carbon was much diminished as judged by the degree of vascular staining. Eventually all the carbon was cleared from the circulating blood.

At necropsy (fig. 1) the organs containing most carbon were the gills and posterior salivary gland both of which became entirely black (fig. 2). The gland of HENSEN (fig. 3) and gill spleen were less deeply pigmented. The anterior salivary gland contained only a small amount of carbon and was greyish white in colour. The liver, kidney, heart, large vessels, muscles, alimentary tract and ovaries showed no pigmentation.

Microscopic examination of the gill after the large dose of carbon reveals an abundance of densely packed phagocytic cells (fig. 4). With a smaller dose (figs. 5 and 6) it is seen that only the stromal phagocytic cells contain carbon and the epithelium has none. The small vessels are patent and show no thrombosis.

The posterior salivary gland (fig. 7) contains large numbers of phagocytic cells within the stroma. These phagocytic cells are intimately mixed with mononuclear leucocytes which do not contain carbon. These mononuclear cells closely resemble the human monocyte.

The anterior salivary gland contains far fewer phagocytic cells than the lower. Occasionally carbon is deposited extracellularly on the basement membranes and collagenous tissue of the acini. Fig. 8 shows carbon deposited beneath the tall columnar cells. Slight to moderate numbers of phagocytic cells were also found in the gill spleen and branchial heart.

The gland of Hensen contained moderate numbers of phagocytic cells within the sinusoids (fig. 9). None were sessile and the solid cords of leucopoietic tissue failed to show any phagocytic activity. Sections through the pouches of the vena cava showed only traces of carbon in a small number of the excretory cells although the capillary endothelial cells had taken up moderate amounts (fig. 10).

The peripheral blood (fig. 11) showed mononuclear cells 12-15 μ in diameter. The cytoplasm contained minute and barely discernible granules. The nucleus was characteristically lobed and very similar to the human monocyte.

DISCUSSION

The carbon used in these experiments was stable in invertebrate blood and did not aggregate into large clumps or cause thrombosis. It is not toxic and large doses can be given without ill effects. Most workers have used either carmine or indian ink for the study of invertebrate phagocytic systems. STAUBER (1950) investigated the fate of India ink injected intracardially into the oyster and TRIPP (1958) used red blood cells of vertebrates for a similar investigation. CHENG and STREISFELD (1963) showed that the haemocytes of the trematode *Megalodiscus temperatus* were capable of phagocytosing indian ink and that the haemocytes were subsequently eliminated by migration into the alimentary tract. BROWN and BROWN (1965) studied the fate of thorium dioxide injected into the pedal sinus of *Bullia*. This material has the advantage of being opaque to x-rays and its distribution can be followed by serial radiographs. Colloidal carbon is not radio-opaque but is readily visible in histological sections. Using techniques devised for mammals by BENACERAFF, BIOZZI, HALPERN and STIFFEL (1956) it would be possible to make quantitative measurements of R.E.S. activity and at the same time observe whether agents which stimulated or depressed the R.E.S. were active at this level of evolution (STUART, 1963).

When insoluble particulate matter is injected into mammals it is stored in the reticulo-endothelial system and may be found in the Kupffer cells of the liver, splenic macrophages and histiocytes of abdominal and thoracic lymph nodes. Excretion is slow and some materials such as thorotrast or carbon are stored for years although some loss occurs by migration of phagocytic cells into the lung. By contrast most invertebrates excrete foreign particulate matter readily. BROWN and BROWN (1965) described in *Bullia* the removal of phagocytic haemocytes which migrated by various routes to the outside of the body. The main pathway was through the heart wall into the pericardial cavity and via the renopericardial canal into the lumen of the kidney from which the cells escaped into the mantle cavity. No such mechanism was observed in *Eledone* where only minute traces of carbon were found in the excretory cells and as long as 14 days after injection an abundance of carbon was still present in various organs.

The phagocytic powers of various tissues can be very roughly graded as follows. Posterior salivary gland and gills > gland of Hensen > anterior salivary gland > branchial spleen. The abundance of phagocytes in the gill can be correlated with the view that this is a protective barrier between the organism and external environment but it is difficult to explain why the salivary gland has so many of these cells. The dense packing of carbon filled cells in the interstices of the gland suggests the presence of a sinusoidal vascular system in which the cells are in direct contact with the blood. This view is supported by the work of GRAZIADEI (1962) who described the anatomy of blood vessels in the posterior salivary gland of *Octopus vulgaris*. He found that the salivary artery, which is a branch of the cephalic aorta, divided inside the gland into a capillary network which supplied the walls of excretory ducts, and into a sinusoidal system which supplied the secretory glandular part of the organ. The two systems, capillary and sinusoidal were intercommunicating.

The function of the gland of Hensen still remains obscure. In a detailed and illustrated study BOLOGNARI (1951) has discussed the physiology of this organ and has suggested that it may also have renal function by degrading purines to xanthine which is subsequently excreted into the blood. CAZAL and BOGORAZE (1943) reviewed the histology of this organ and supported the view that it formed leucocytes. They injected carmine which was taken up by large cells with vacuolated nuclei and basophilic cytoplasm. These cells are almost certainly the same as the phagocytic cells detected with colloidal carbon and the present work confirms this earlier observation by these authors. NECCO and MARTIN (1963) were able to grow the cells of the white body in tissue culture and noted two types of cells, one spindle shaped and the other rounded. This accords with the present observations which show both phagocytic cells and non-phagocytic leukoblastic cells. Many of the latter resemble monocytes and this is the dominant cell in the peripheral blood. BARBER and GRAZIADEI (1965) made an electron microscopic study of the blood of cephalopods and apart from amoebocytes noted no other type of cell. Their amoebocytes had numerous mitochondria in the cytoplasm, an endoplasmic reticulum with ribosomes and also large membrane bound granules approximately 0.5 μ in diameter. It is not known whether these correspond to the avidly phagocytic cells found in the present study.

It now seems clear that the octopus has an extensive reticulo-endothelial apparatus and it would be surprising if it did not contain a least a primitive " recognition system " for foreign materials. Phagocytic activity is greatly enhanced if the ingested particles are coated with globulin and the theory is put forwards

that the acquisition during evolution of globulin forming cells had as its main function the facilitation of phagocytosis by the reticulo-endothelial system. Since a conspicuous feature of the octopus is a lack of lymphatics and discrete foci of lymphoid tissue the problem arises as to whether it is able to opsonise foreign substances. Because of this lack of cellular sophistication the octopus offers a suitable experimental model for studies in immunology.

SUMMARY

The reticulo-endothelial apparatus of *Eledone cirrosa* has been investigated by the intravenous injection of specially prepared colloidal carbon. Phagocytic cells were found in the posterior salivary gland and gills, the white body or gland of Hensen, anterior salivary gland and blanchial spleen. In the gland of Hensen both phagocytic and non phagocytic leukoblastic cells were found. These cells resemble monocytes and it is suggested that this organ may be responsible for a primitive recognition system useful in basic immunological responses.

RÉSUMÉ

Le système réticulo-endothélial de *Eledone cirrosa* a été étudié par injection intraveineuse d'une préparation spéciale de carbone colloïdal. Des cellules phagocytaires ont été trouvées dans la glande salivaire postérieure et les branchies, le « corps blanc » ou glande de Hensen, la glande salivaire antérieure et la glande branchiale. Des cellules leucoblastiques phagocytaires et non phagocytaires ont été trouvées dans la glande de Hensen. Ces cellules ressemblent aux monocytes, et il est possible que cet organe constitue un système primitif de reconnaissance utile dans les réponses immunologiques de base.

ZUSSAMENFASSUNG

Das reticulo-endotheliale System von *Eledone cirrosa* wurde an Hand von intravenösen Einspitzungen eines speziell zubereiteten, gallertartigen Kohlenstoffes untersucht. Phagocyten sind in der

hinteren Speicheldrüse, den Kiemen, im weissen Körper, auch Hensen'sche Drüse genannt, in der vorderen Speicheldrüse und in der Kiemendrüse gefunden worden. Phagocytäre und nicht phagocytäre Leucoblasten wurden in der Hensen-schen Drüse entdeckt. Diese Zellen gleichen den Monocyten; es wird vermutet, dass der weisse Körper ein enfaches Erkennungssystem darstellt, das brauchbar ist für fondamentale immunologische Antworten.

REFERENCES

BARBER, V.C. and P. GRAZIADEI, 1965. The fine structure of cephalopod blood vessels. *Zeit. f. Zellforsch.*, **66** : 765-781.

BENACERAFF, B., G. BIOZZI and B.N. HALPERN, 1956. *In* Physiopathology of the reticulo-endothelial system. Edit. B.N. Halpern, Blackwell, Oxford.

BOLOGNARI, A., 1951. Mofolofia, struttura e funzione del « corpo bianco » dei Cefalopodi. *Arch. Zool. Ital.*, **XXXVI**, 253-285.

BROWN, A.C. and R.J. BROWN, 1965. The fate of thorium dioxide injected into the pedal sinus of Bullia (Gastropoda : Prosobranchiata). *J. Exp. Biol.*, **42** : 509-519.

CAZAL, P. and D. BOGORAZE, 1943. Recherches sur les corps blancs du Poulpe (*Octopus vulgaris* Lam.). Leur fonction globuligène et néphrocytaire. *Bull. de l'Institut Océanographique*, n° **842**, 29 avril.

CHENG, T.C. and S.D. STREISFELD, 1963. Innate Phagocytosis in the Trematodes Megalodiscus temperatus and Haematoloechus sp. *J. Morph.*, **113**, n° 3 : 375-380.

GRAZIADEI, P., 1962. Sulla vascolarizzione e sulla innervazione delle ghiandole salivari posteriori di *Octopus vulgaris*. *Rivista di Biologica*, **LV** (4) : 385-395.

ISGROVE, A., 1909. L.M.B.C. Memoirs. XVIII Eledone pub. Williams and Norgate, London.

NECCO, A. and R. MARTIN, 1963. Behaviour and estimation of the mitotic activity of the white body cells in *Octopus vulgaris*, cultured in vitro. *Exp. Cell Res.*, **30** : 588-623.

STAUBER, L.A., 1950. The fate of india ink injected intracardially into the oyster, ostrea virginia gmelin. *Biological Bull.*, *98*, 227-241.

STUART, A.E., 1963. Structural and Functional Effects of lipids on the reticulo-endothelial system. Colloq. Int. du Centre National de la Recherche Scientifique, n° **115**, 129-146.

TRIPP, M.R., 1958. Disposal by the oyster of intracardially injected red blood cells of vertebrates. *Proc. Nat. Shellfisheries Associa.*, **48**, 148-147.

YOUNG, J.Z., 1964. A model of the Brain. Oxford, Clarendon Press.

Reçu le 11 mai 1966.

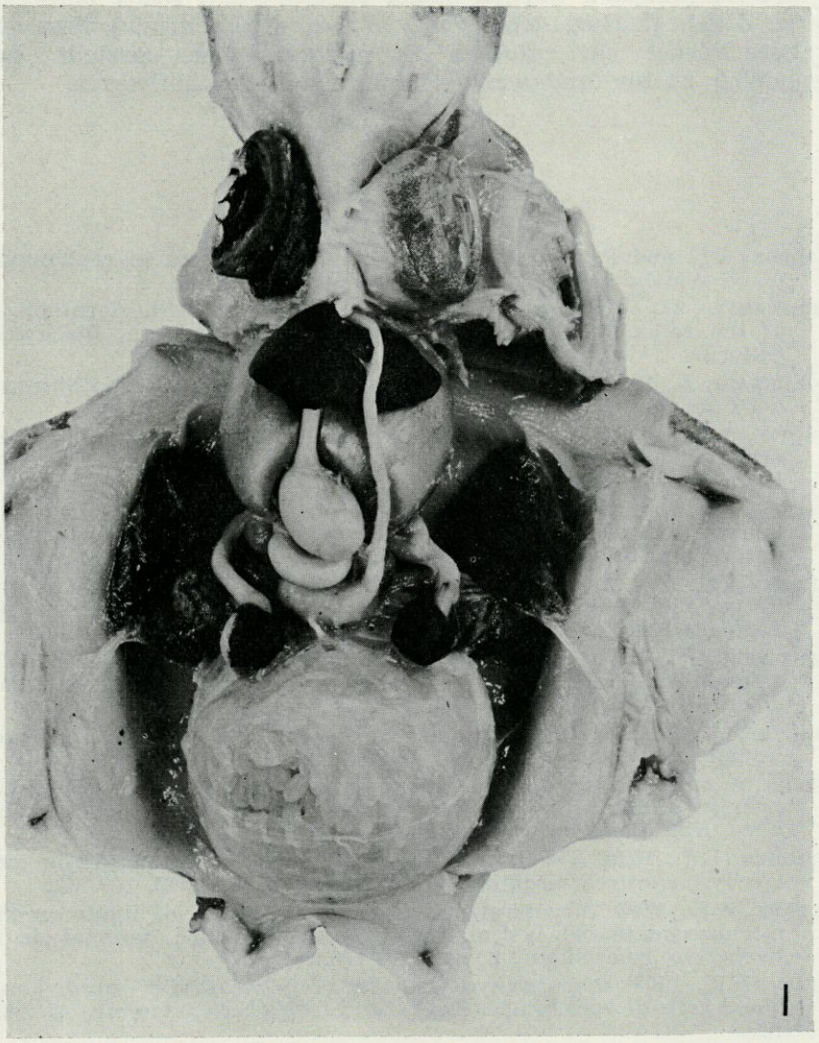
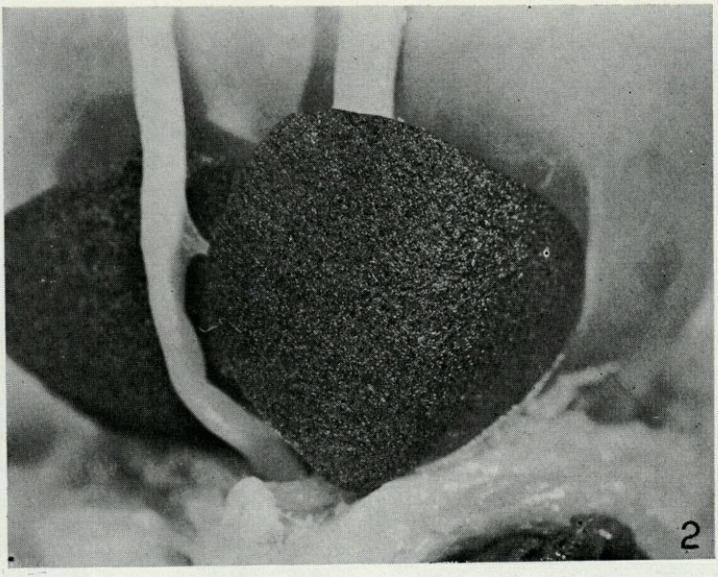


FIG. 1. — Dissection made 2 days after injection of high dose of colloidal carbon. Organs containing phagocytic cells appear black.



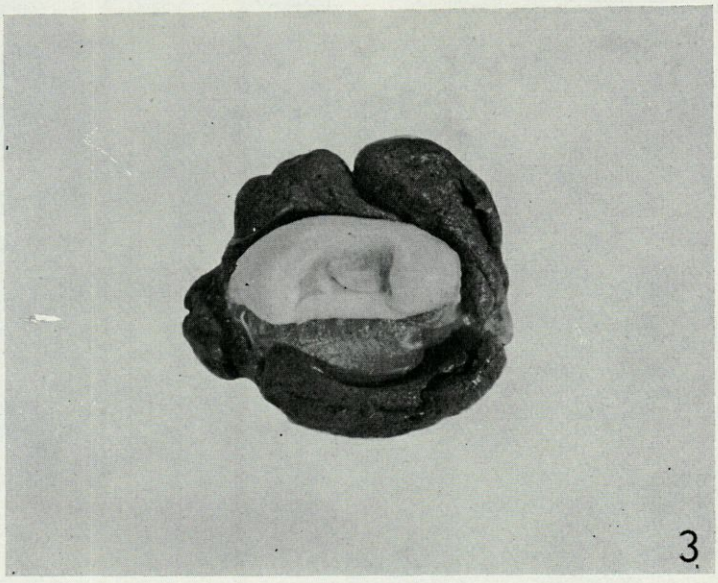
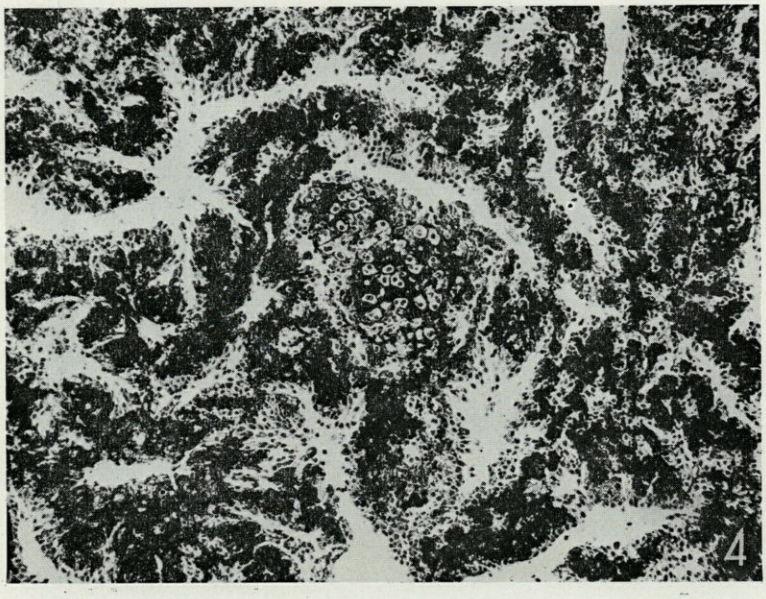


Fig. 2. — Posterior salivery gland after intravenous injection of carbon. The natural colour is white; note the black appearance (enlarged from fig. 1).

Fig. 3. — The gland of Hensen or Faussek after carbon. The central pale structure is the optic ganglion, the surrounding collar of dark tissue represents the lobes of the gland which is normally white.



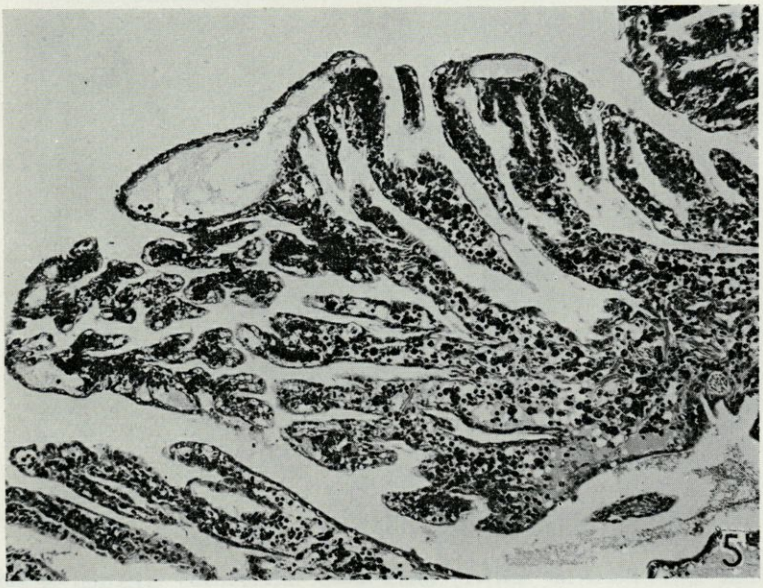
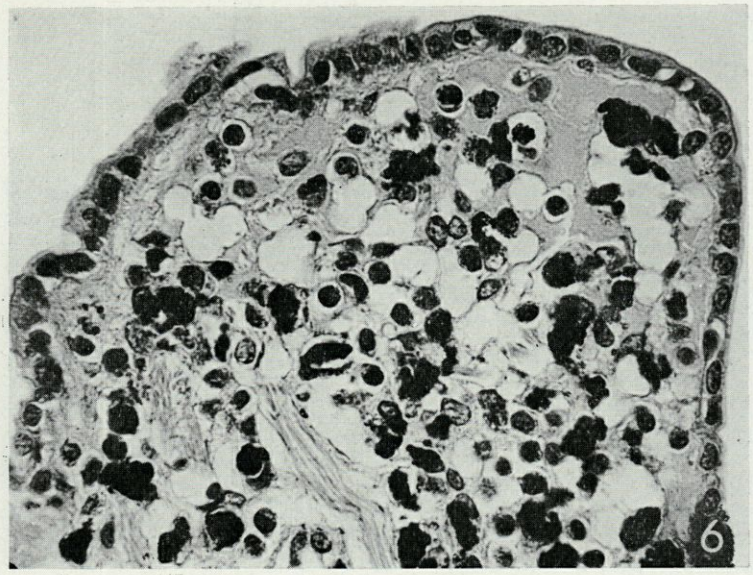


Fig. 4. — Gill after large dose of carbon. H. & E. × 100.

Fig. 5. — Gill after small dose of carbon. H. & E. × 100.



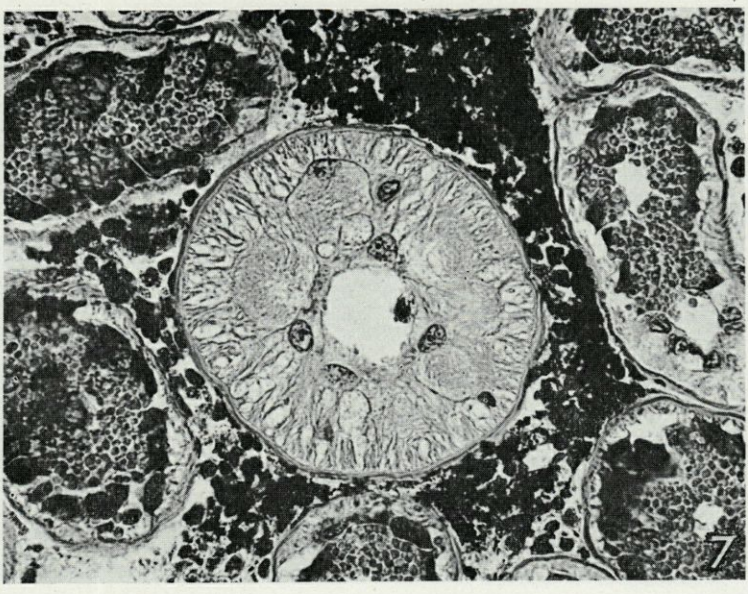
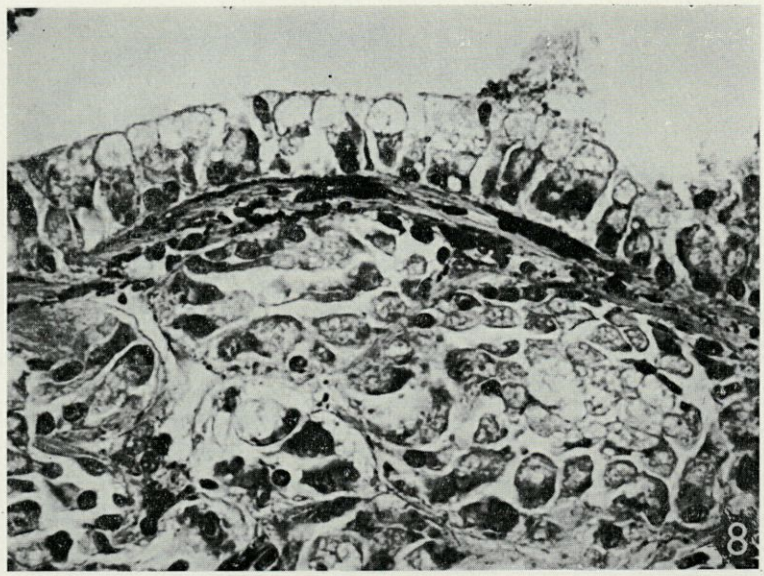


Fig. 6. — Gill after small dose of carbon. H. & E. × 550.
Note abundance of deeply pigmented stromal phagocytes.

Fig. 7. — Posterior salivary gland showing abundant stromal phagocytic cells. H. & E. × 400.



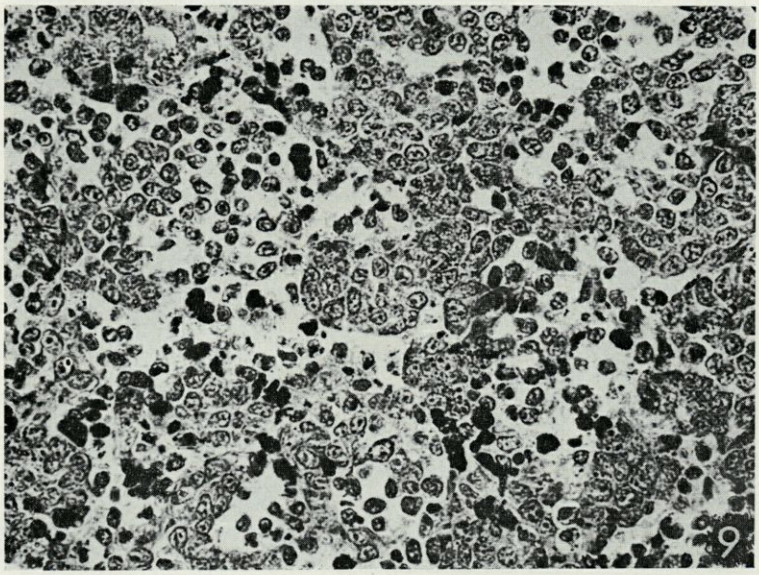
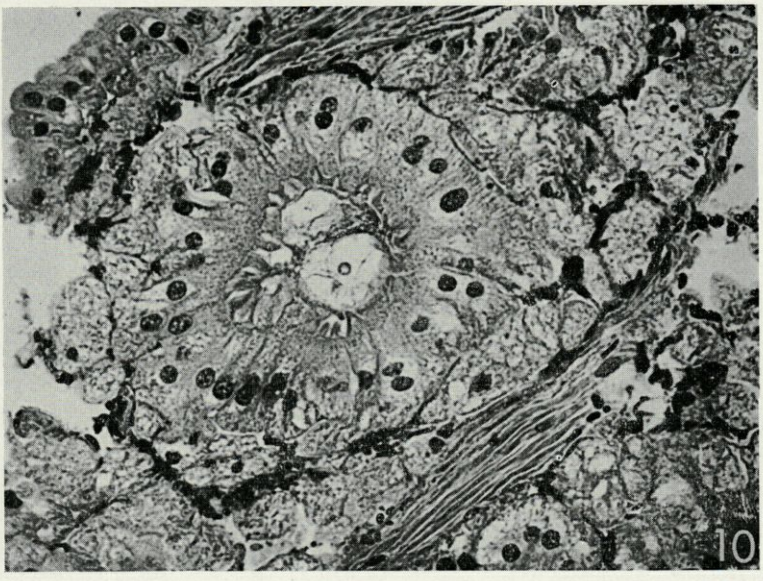


Fig. 8. — Anterior salivary gland showing anomolous deposition of carbon on collagenous fibres beneath the columnar epithelium. H. & E. × 500.

Fig. 9. — Gland of Hensen. The carbon containing phagocytic cells are lying free within sinusoidal channels bounded by solid cords of leucoblastic tissue. H. & E. × 425.



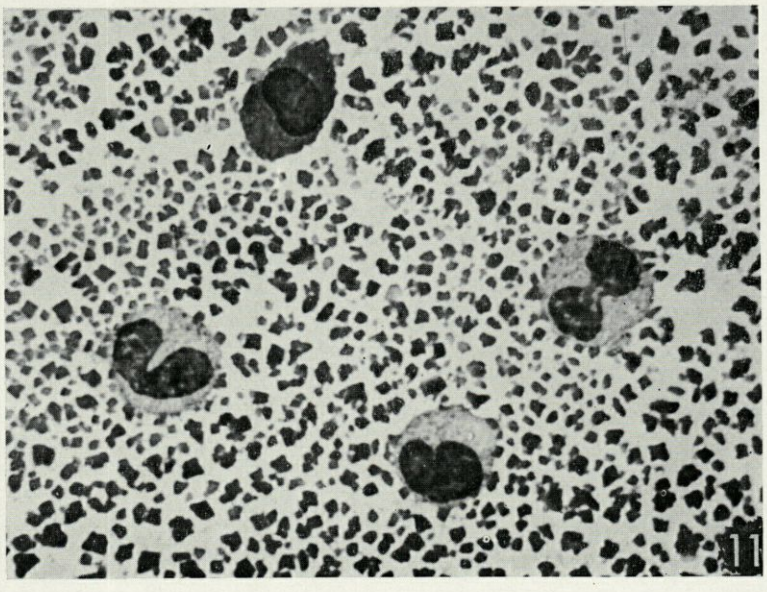


Fig. 10. — Section through pouches of vena cava after high dose of carbon showing absence of carbon in secretory celles and small amounts in endothelial cells. H. & E. × 300.

Fig. 11. — Peripheral blood. × 1000 (Leishman). Note resemblance of nuclei to human monocytes.